PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43*bis*.1)

Applicant's or agent's file reference see form PCT/ISA/220	Date of mailing *(day/month/year)* see form PCT/ISA/210 (second sheet) **FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/CA2004/000998	08.07.2004	08.07.2003

International Patent Classification (IPC) or both national classification and IPC
C12N5/06, A01K67/027

Applicant
MCGILL UNIVERSITY

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/CA2004/000998

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was field, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☐ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☐ in written format

   ☐ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/CA2004/000998

Box No. II Priority

1. ☒ The following document has not been furnished:

   ☒ copy of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(a)).

   ☐ translation of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(b)).

   Consequently it has not been possible to consider the validity of the priority claim. This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1,2,4,5,14-19
	No:	Claims	3,6-13,20-22
Inventive step (IS)	Yes:	Claims	-
	No:	Claims	1-22
Industrial applicability (IA)	Yes:	Claims	1-22
	No:	Claims	-

2. Citations and explanations

see separate sheet

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY (SEPARATE SHEET)

International application No.

PCT/CA2004/000998

The present application relates to embryonic stem (ES) cells obtainable from crosses between C57BL/6 and 129 inbred mouse (sub)strains. Some of said ES cells comprise a transgene docking site for introduction of a single copy introduction of a transgene. It has been shown that some of the ES cells derived from three inbred strains had the potential to give rise to chimeras with 100% ES cell derived coat color using the morula aggregation technique of generating embryos, indicating good development potential.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1) Reference is made to the following documents:

D1: EGGAN KEVIN ET AL: "Hybrid vigor, fetal overgrowth, and viability of mice derived by nuclear cloning and tetraploid embryo complementation" PROC. NATL. ACAD. SCI. USA, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, US, vol. 98, no. 11, 22 May 2001 (2001-05-22), pages 6209-6214

D2: YAGI TAKESHI ET AL: "A novel ES cell line, TT2, with high germline-differentiating potency" ANALYTICAL BIOCHEMISTRY, vol. 214, no. 1, 1993, pages 70-76

D3: JASIN ET AL: "Targeted transgenesis" PROC. NATL. ACAD. SCI. USA, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, US, vol. 93, no. 17, August 1996 (1996-08), pages 8804-8808

NOVELTY (Article 33(2) PCT)

2.1) D1 discloses ES cells derived from various crosses between two different inbred mouse strains and shows that said F_1 ES cell clones can give rise to embryos entirely (100%) derived from said ES cells, when injected into tetraploid blastocysts and subsequently transferred to recipient females. Some of the ES cells described were derived from mouse strains carrying a transgene docking site, the *Rosa26* locus, and mice have been generated from said cells after targeting of said *Rosa26* locus (D1, table 4).

D1 therefore anticipates the subject-matter of claims 3, 6-13 and 20-22 (Article 33(2)

PCT).

2.2) In addition, claims 12 and 13 also lack novelty because a product is not rendered novel merely by the fact that it is produced by means of a new process (PCT Guidelines A5.26[1]), and because there are no technical features present in claims 12 and 13 that allow the skilled person to distinguish between an ES cell derived mouse or transgenic mouse of said claims from (transgenic) mice obtained by crossing two or three inbred strains of mice known in the art (Article 33(2) PCT).

2.3) The subject-matter of claims 1, 2, 4, 14-19 is not disclosed in the prior art, therefore said claims are novel (Article 33(2) PCT).

INVENTIVE STEP (Article 33(3) PCT)

3.1) The document D2 is regarded as being the closest prior art to the subject-matter of claim 1, and discloses (D2, table 1): ES cells derived from a cross between two different inbred mouse strains, that give rise to chimeras that are 100% ES cell derived upon injection of the ES cells into eight-cell stage embryos.
From this the subject-matter of claim 1 differs in that **three** inbred strains have been used to generate ES cells having maximal heterosis and related development potential.

The problem to be solved by the present invention may therefore be regarded as the provision of further ES cells with maximal heterosis.

No effect has been indicated of the use ES cells derived from a cross between three different inbred mouse (sub)strains compared to ES cells derived from a cross between two inbred mouse strains for the ability of said ES cells to contribute at a high percentage to the embryos generated from said ES cells, since the ES cells disclosed in D2 give rise to entirely ES cell derived embryos (D2, table 1).

Therefore no inventive step may be acknowledged for the subject-matter of claim 1 (Article 33(3) PCT).

3.2) Similarly, no effect has been indicated for the subject-matter of claims 14 and 15, which relate to ES cells obtained after multiple generations of breeding following the initial cross between two (claim 14) or three (claim 15) inbred mouse strains, without indication how the subsequent (back)crosses affect the capacity of the ES cells to

contribute to chimeras resulting from said ES cells.

3.3) Dependent claims 2, 4, 5 and 16-19 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step, the reasons being as follows:
the use of a specific sequence allowing introduction of a single copy insertion of a transgene in such a manner that does not disrupt endogenous genes, such as a deletion mutant of an X-linked hypoxanthine phosphoribosyltransferase gene or a sequence comprising a loxP site is well known (D3).
No effect has been indicated for the number of backcrosses used to generate the ES cells on the capacity of the resulting ES cells to contribute to chimeras resulting from said ES cells.

3.4) No inventive step has been acknowledged for the subject-matter of the present set of claims (Article 33(3) PCT).

ARTICLE 6 PCT

4.1) It seems that the ES cell lines disclosed in the application are derived from C57BL/6 and two 129 substrains and that they bear 'unique combinations of C57BL/6 and 129 alleles' (description page 23, lines 4-6). This may be understood to mean that said ES cells comprise alleles of only two inbred mouse strains, rather than alleles of three inbred mouse strains. Therefore no support seem to be provided for an ES cell line comprising alleles derived from at least three inbred mouse strains (claims 1, 2, 15 and claims referring to said claims).

4.2) It is not clear what the essential technical features of the present invention are, since the independent claims refer to ES cells (i) derived from either two or three inbred mouse strains, (ii) with or without transgene docking site, and (iii) with or without indication of (back)crosses following the initial cross between the inbred strains. A set of claims which does not comprise all essential technical features in all independent claims does not fulfil the requirement of Rule 13.1 PCT with respect to unity of invention.

4.3) The relative term 'good development potential' (claims 1, 3, 10(d), 14, 15) lacks

clarity and may be defined more precisely by technical features that allow comparison with the prior art.

4.4) The term 'transgene docking site' (claims 2, 3, 10(b), 17) has no well recognised meaning in the art and may comprise any known sequence in the genome.

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